Figure 1

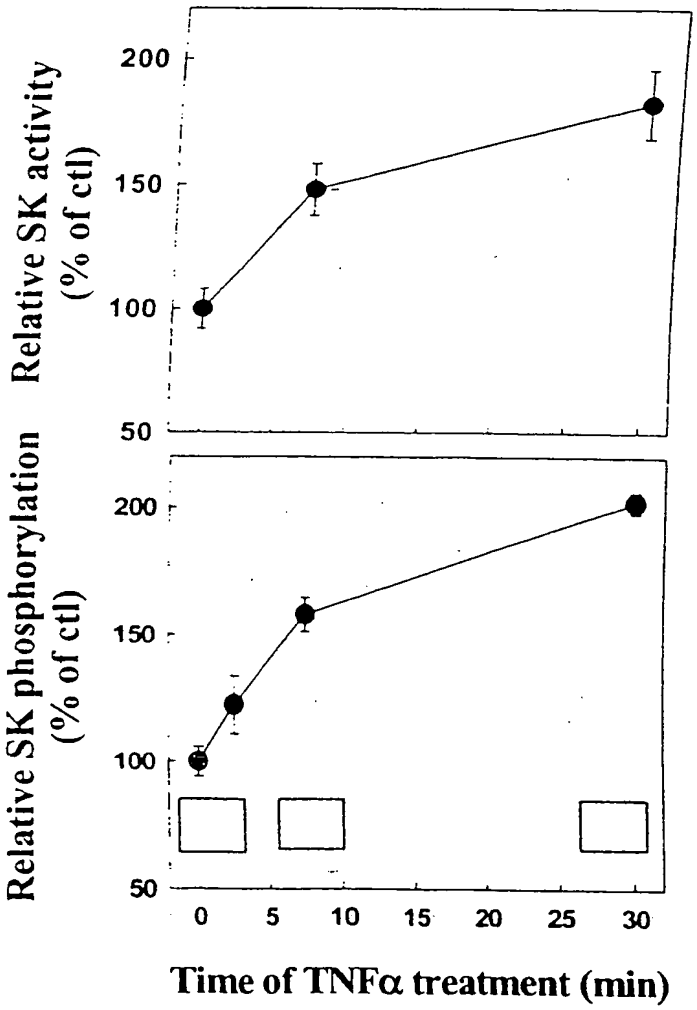
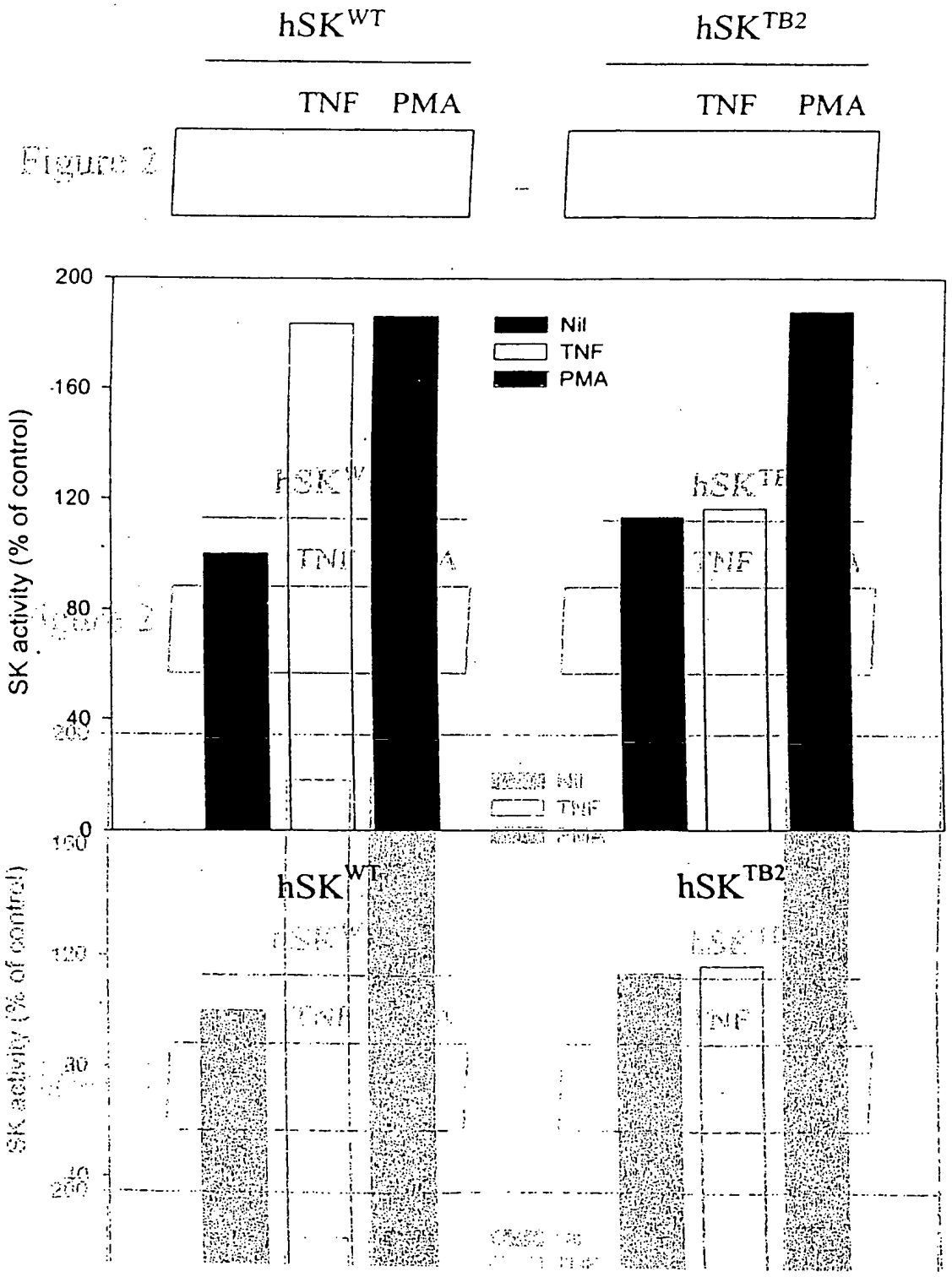

Relative SK activity (% of ctl)
200
150
100
50
Relative SK phosphorylation (% of ctl)
200
150
100
50
0
5
10
15
20
25
30
Time of TNFα treatment (min)

Figure 2



10/509036

Figure 3

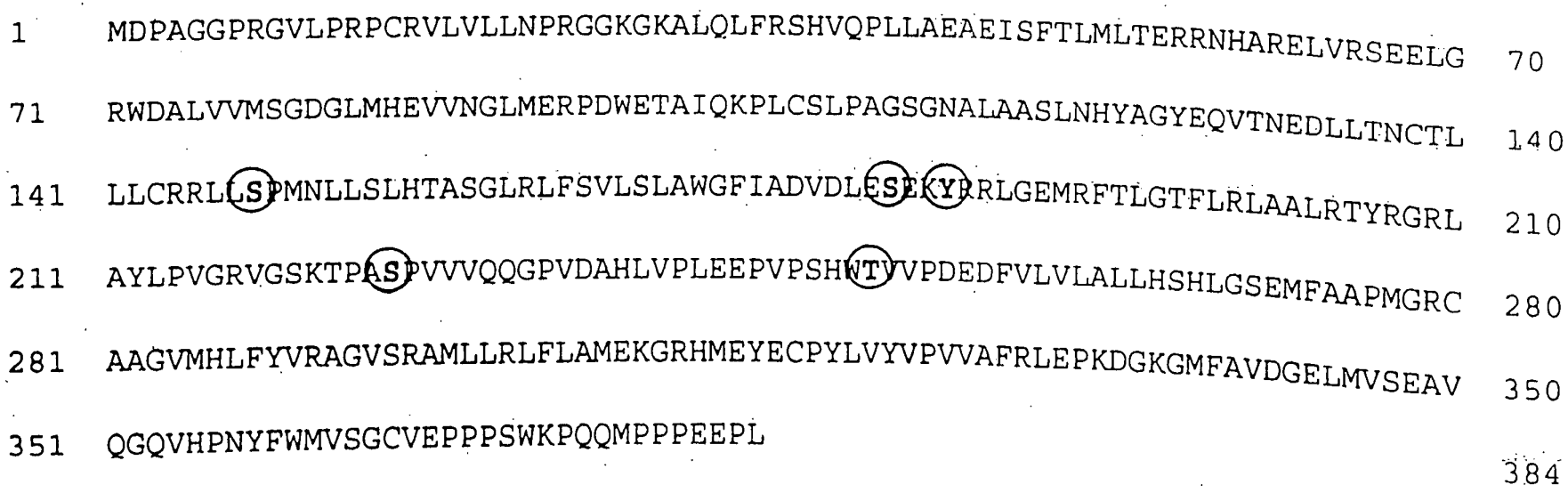


Figure 4

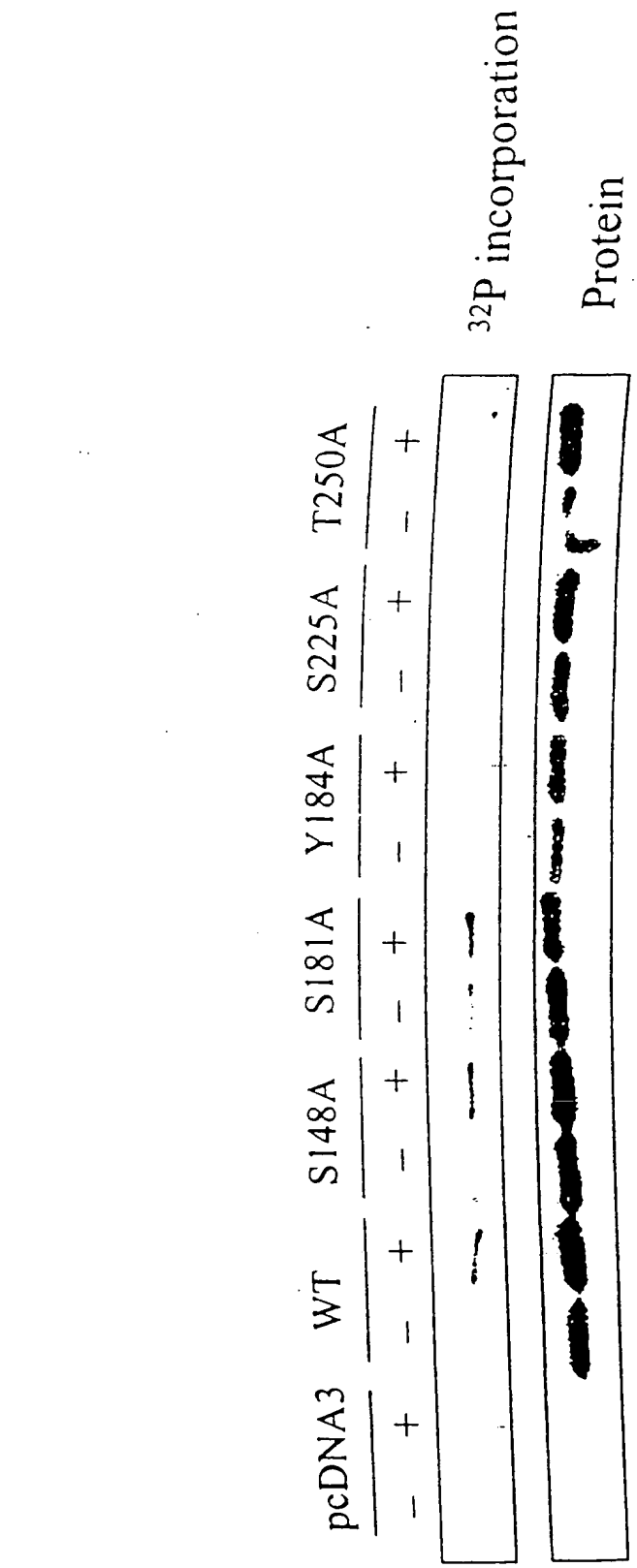


Figure 5

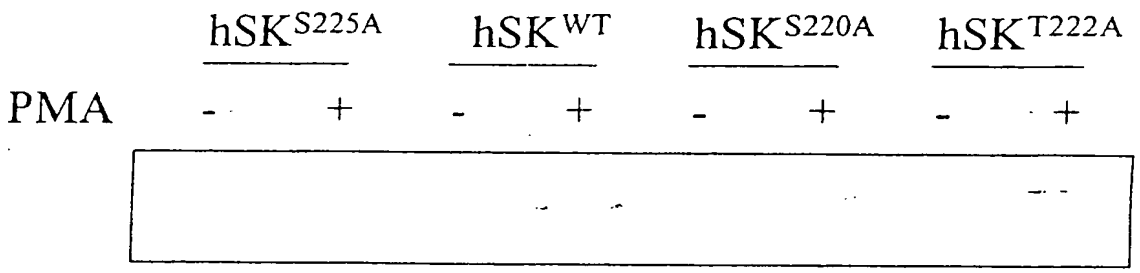


Figure 6

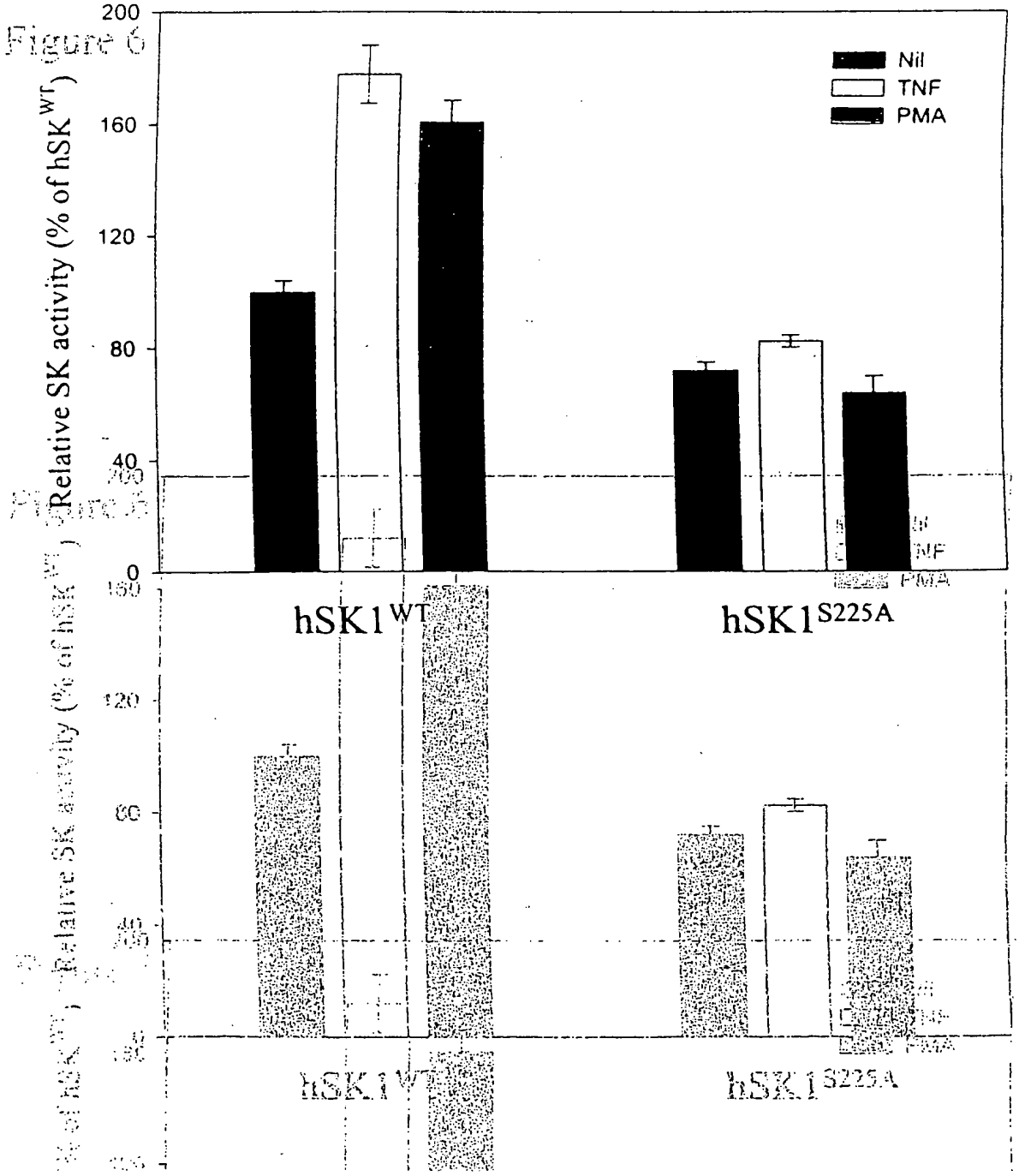

Relative SK activity (% of hSK^{WT})
200
160
120
80
40
0
Nil
TNF
PMA
hSK1^{WT}
hSK1^{S225A}

Figure 7

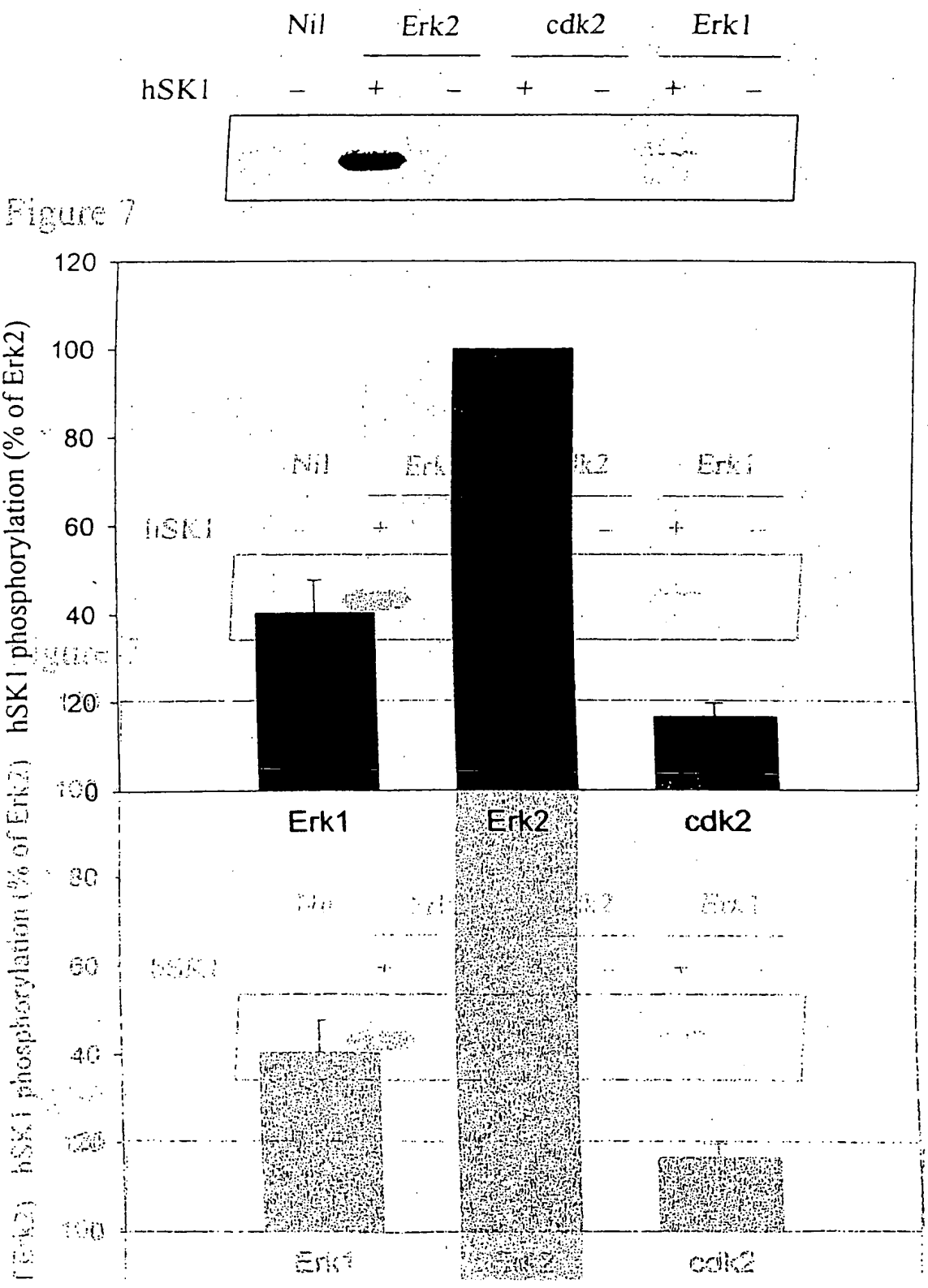


Figure 8

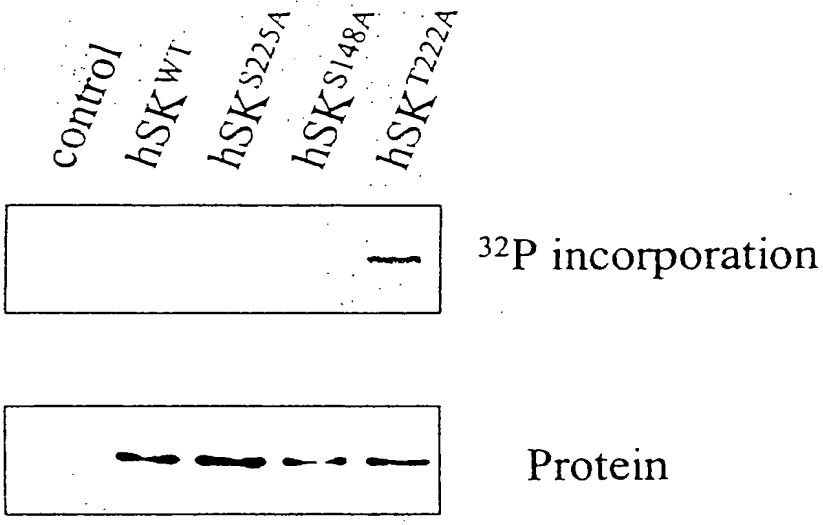


Figure 9

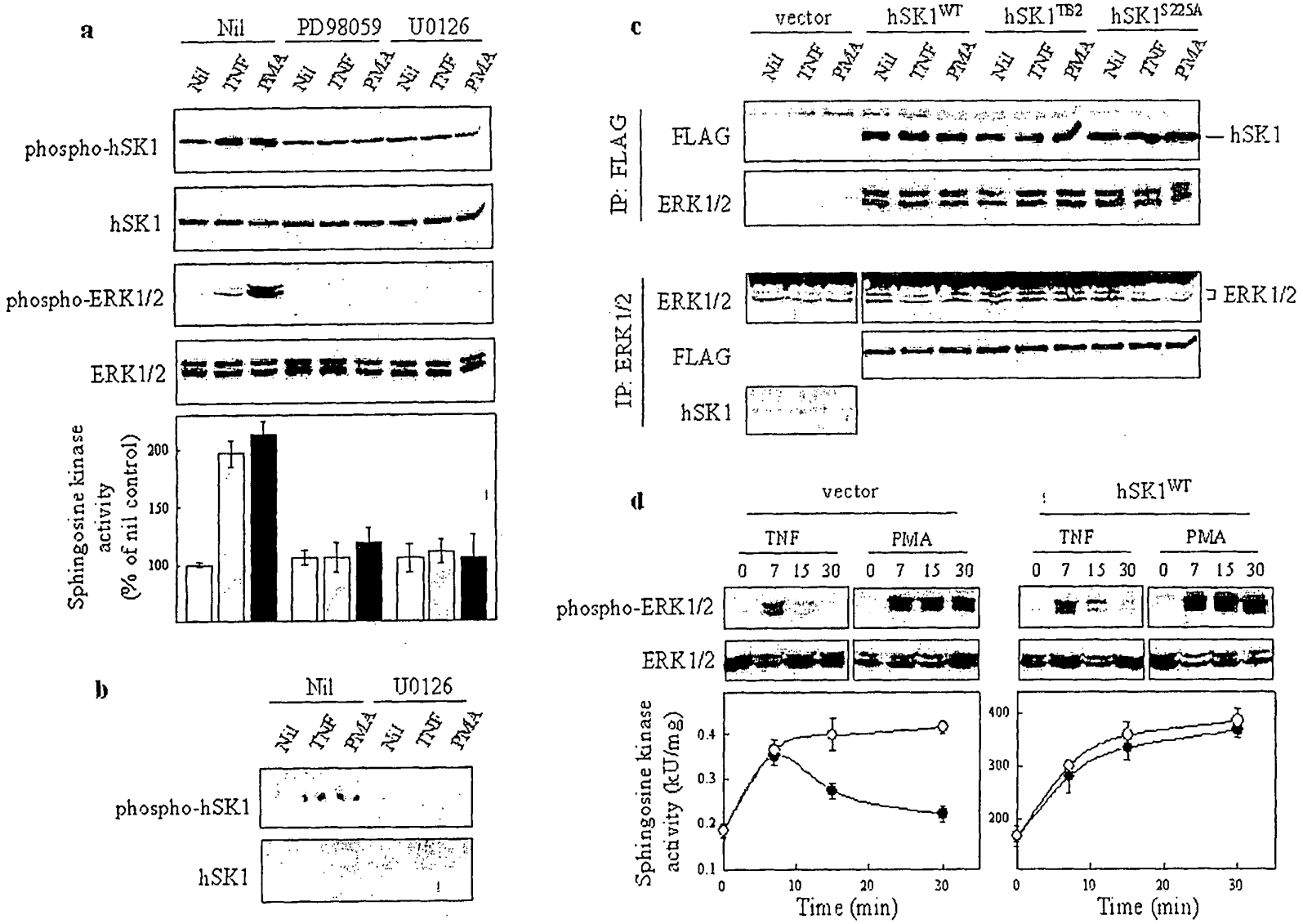

a
Nil
PD98059
U0126
Nil TNF PMA Nil TNF PMA Nil TNF PMA
phospho-hSK1
hSK1
phospho-ERK1/2
ERK1/2
Sphingosine kinase activity (% of nil control)
200
150
100
b
Nil
U0126
Nil TNF PMA Nil TNF PMA
phospho-hSK1
hSK1
c
vector
hSK1WT
hSK1TB2
hSK1S225A
Nil TNF PMA Nil TNF PMA Nil TNF PMA Nil TNF PMA
IP: FLAG
FLAG
hSK1
ERK1/2
IP: ERK1/2
ERK1/2
ERK1/2
FLAG
hSK1
d
vector
hSK1WT
TNF
PMA
0 7 15 30 0 7 15 30 0 7 15 30 0 7 15 30
phospho-ERK1/2
ERK1/2
Sphingosine kinase activity (kU/mg)
0.4
0.3
0.2
0.1
0 10 20 30
Time (min)
400
300
200
0 10 20 30
Time (min)

Figure 10A

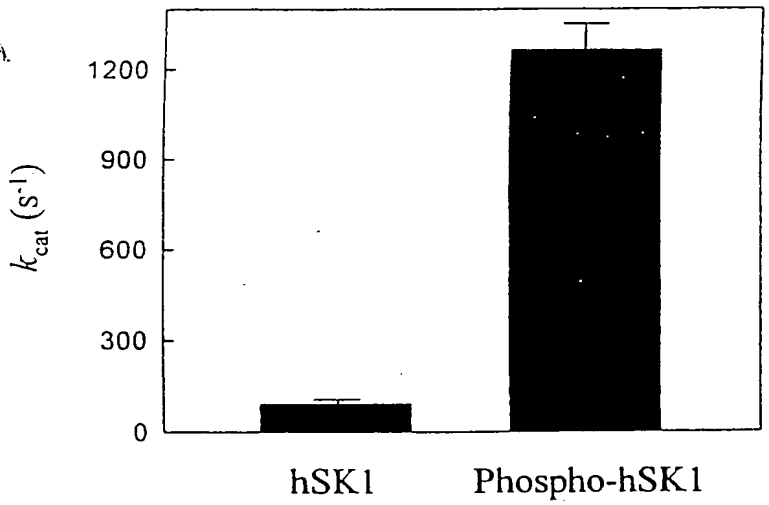


Figure 10A

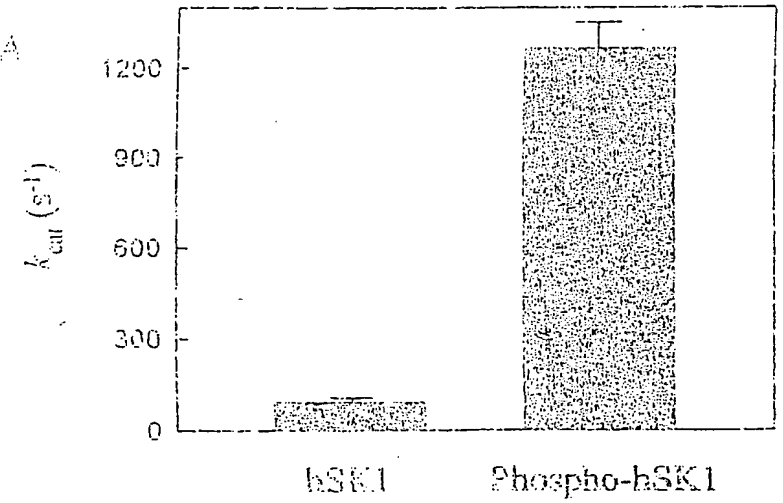


Figure 10A

Figure 10B

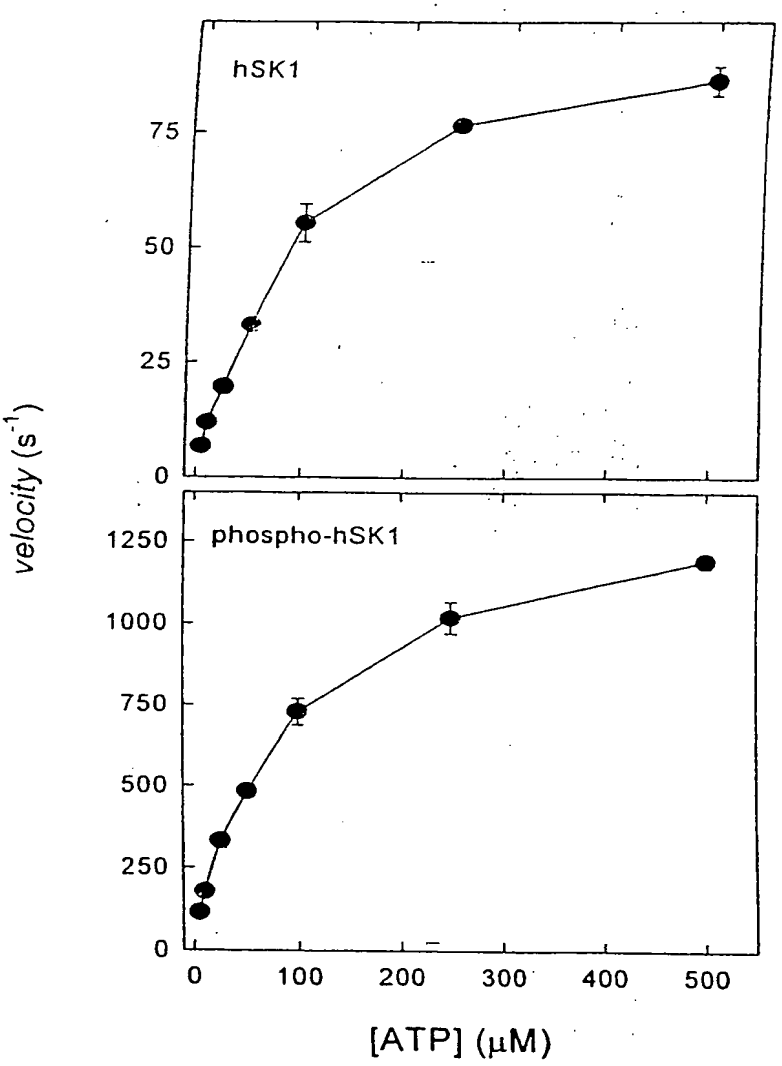

hSK1
phospho-hSK1
velocity (s-1)
0
25
50
75
250
500
750
1000
1250
0
100
200
300
400
500
[ATP] (μM)

Figure 10C

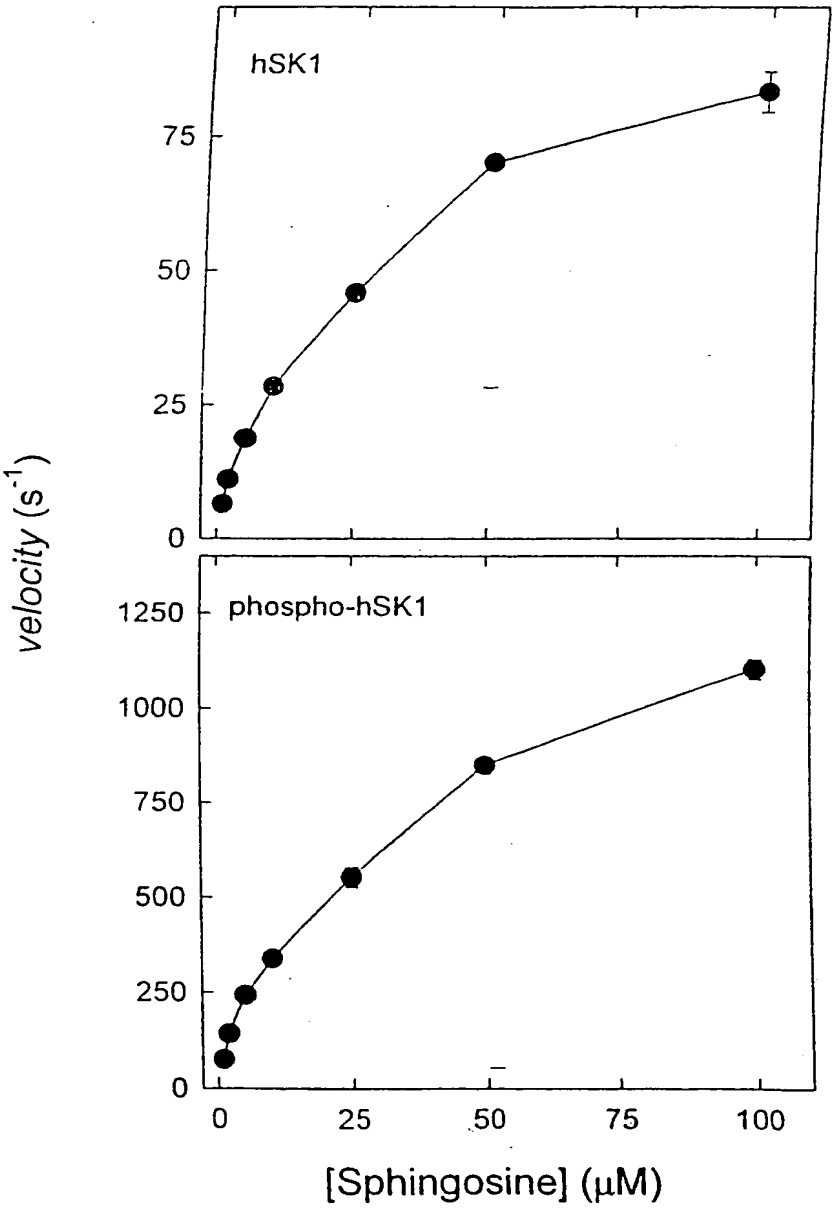

hSK1
phospho-hSK1
velocity (s^{-1})
0
25
50
75
250
500
750
1000
1250
0
25
50
75
100
[Sphingosine] (μM)

Figure 11

Figure 11

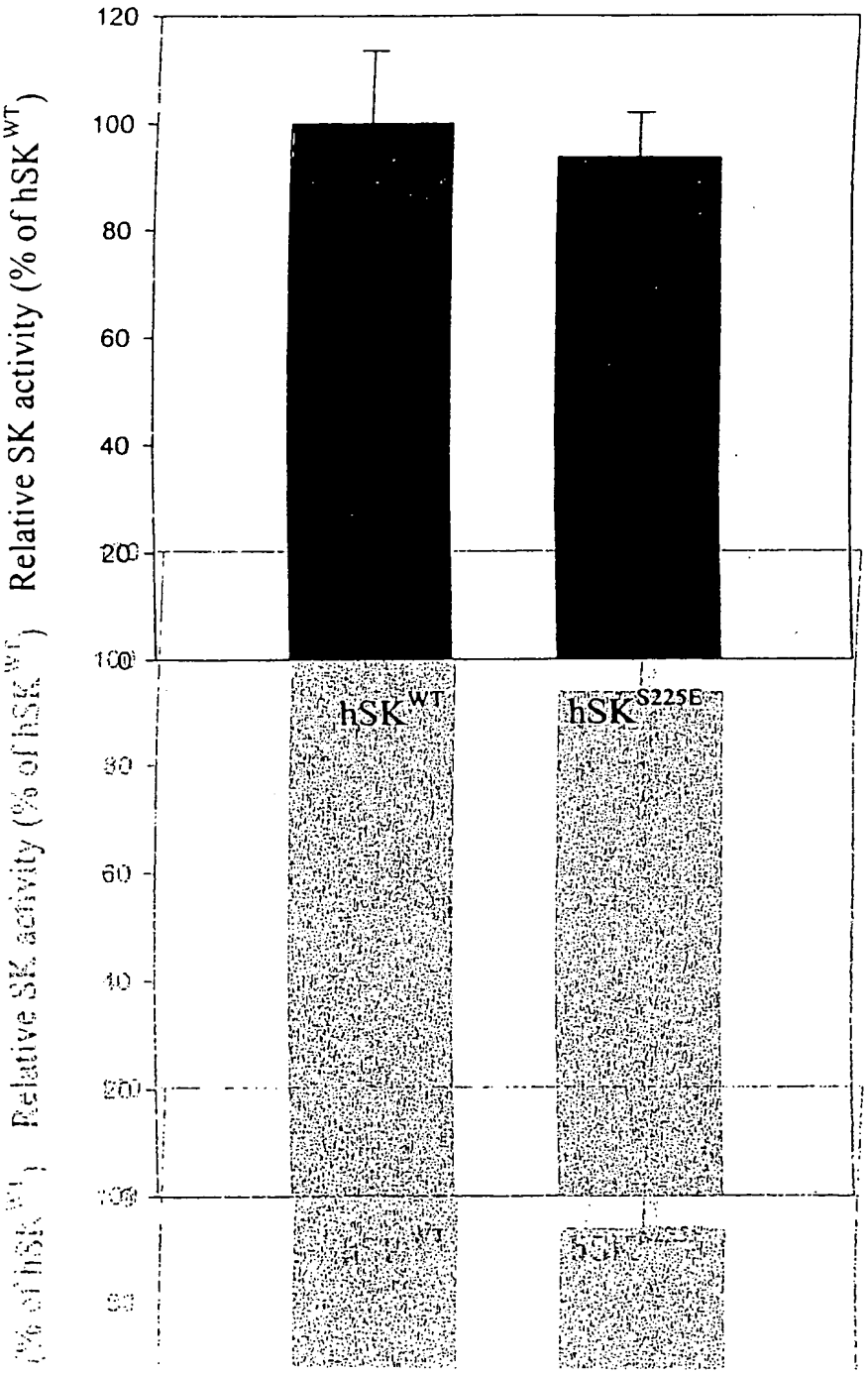


Figure 11

Figure 11

Figure 12

antagonists directly targeting the catalytic activity
(eg. Drugs targeting ATP or Sphingosine binding sites)
ATP
Sphingosine
hSK1
antagonists targeting activation
Protein kinase
(Erk2?)

Figure 13

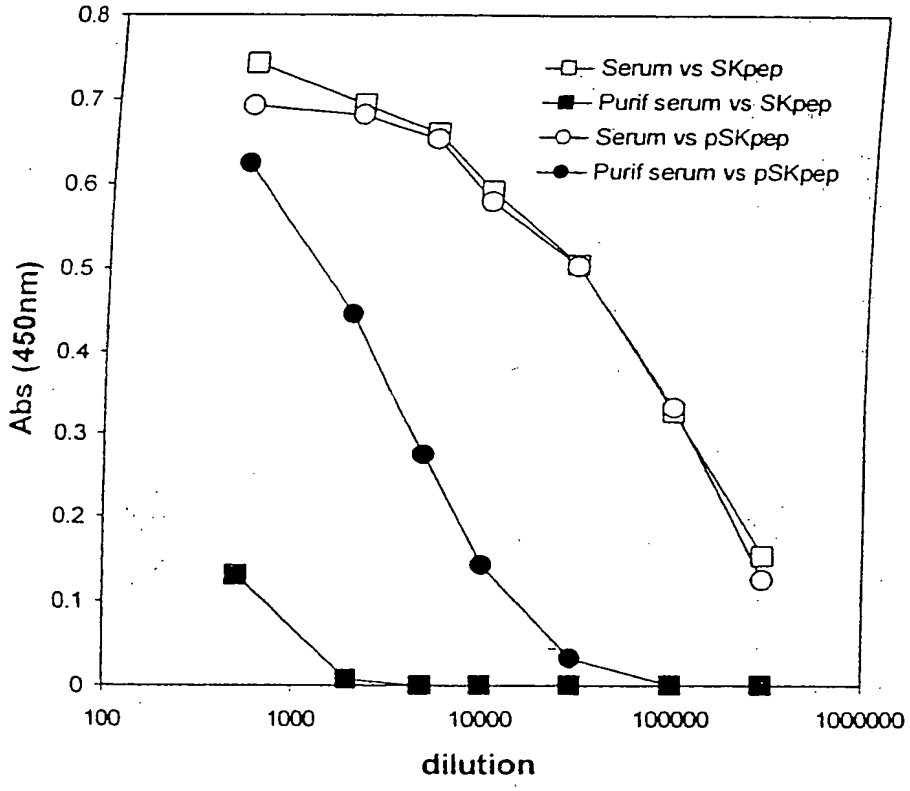


Figure 14

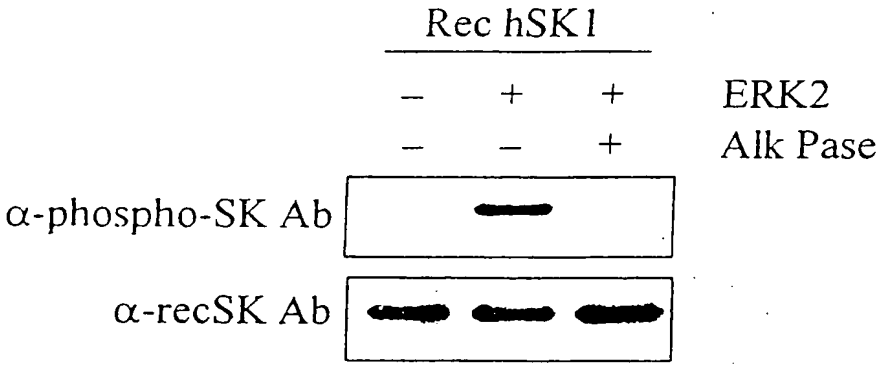


Figure 15

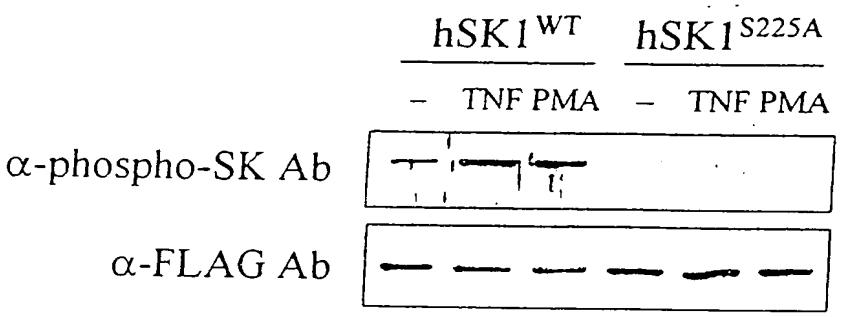


Figure 16

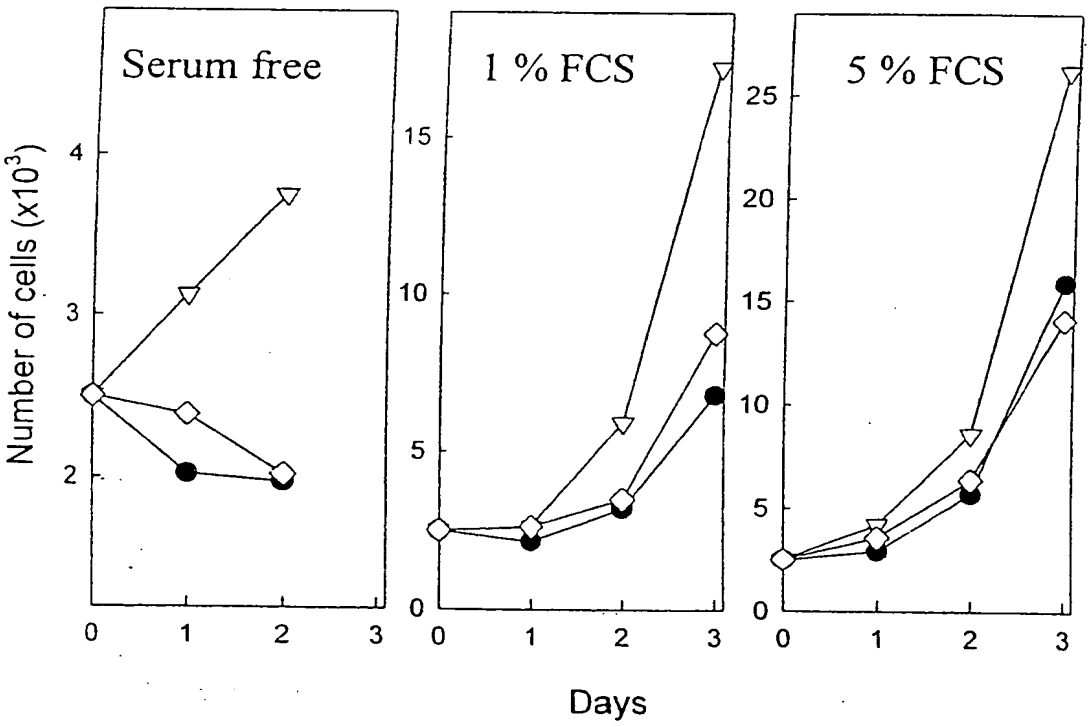
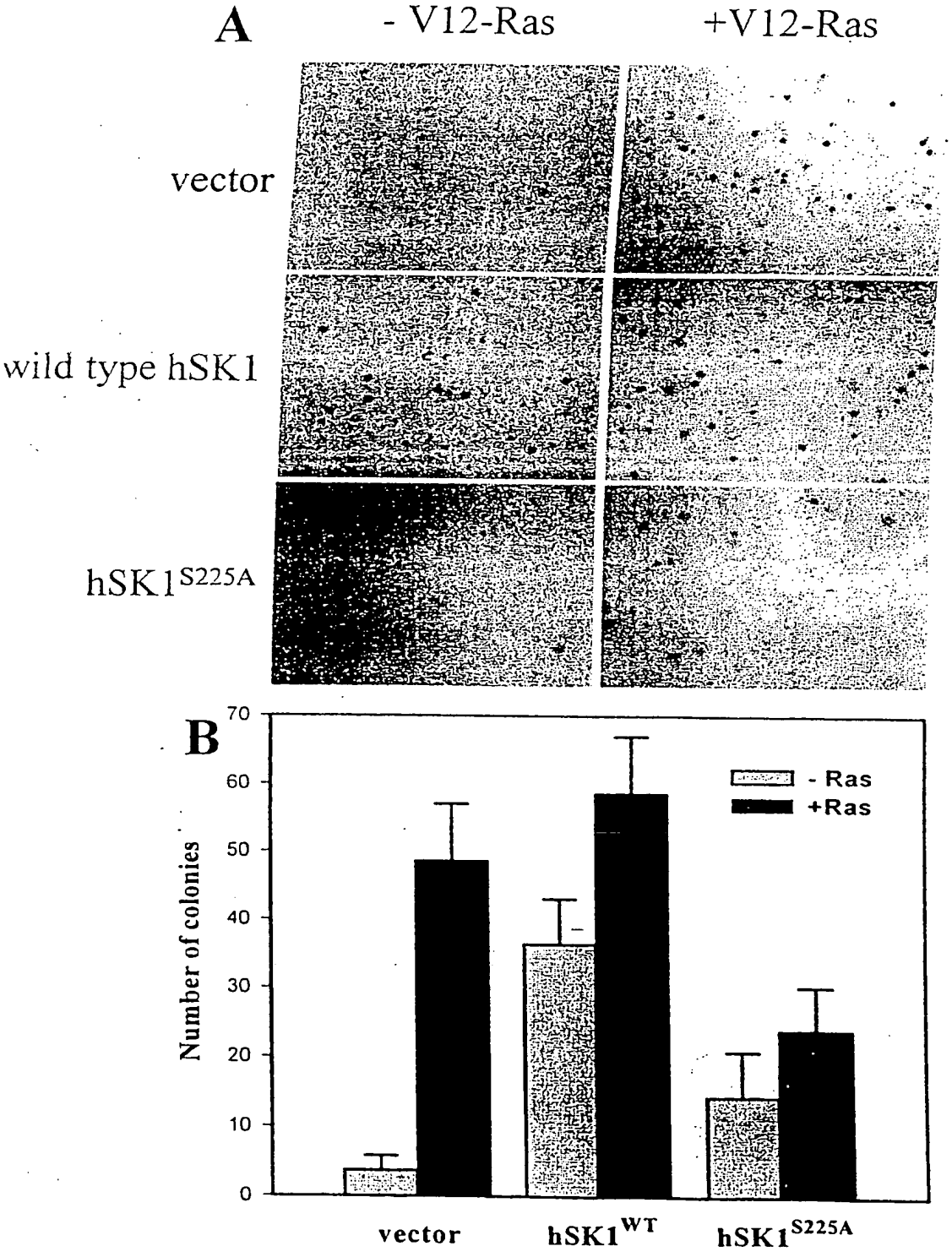


Figure 17


A
- V12-Ras
+V12-Ras
vector
wild type hSK1
hSK1S225A
B
Number of colonies
70
60
50
40
30
20
10
0
- Ras
+Ras
vector
hSK1WT
hSK1S225A

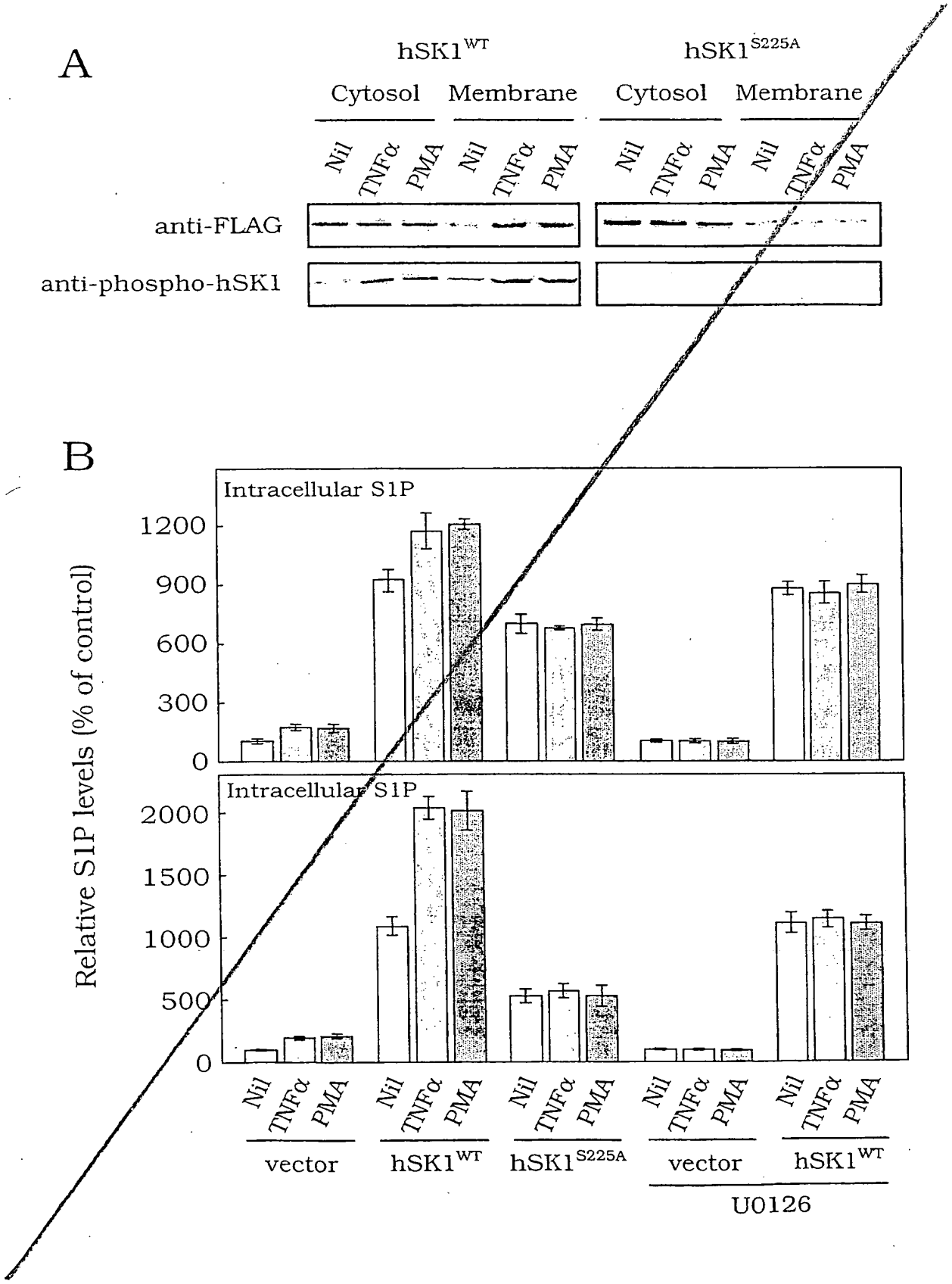

A
hSK1WT
hSK1S225A
Cytosol
Membrane
Cytosol
Membrane
Nil
TNFα
PMA
Nil
TNFα
PMA
Nil
TNFα
PMA
Nil
TNFα
PMA
anti-FLAG
anti-phospho-hSK1
B
Intracellular S1P
1200
900
600
300
0
Intracellular S1P
2000
1500
1000
500
0
Relative S1P levels (% of control)
Nil
TNFα
PMA
vector
hSK1WT
hSK1S225A
vector
hSK1WT
U0126

Figure 18

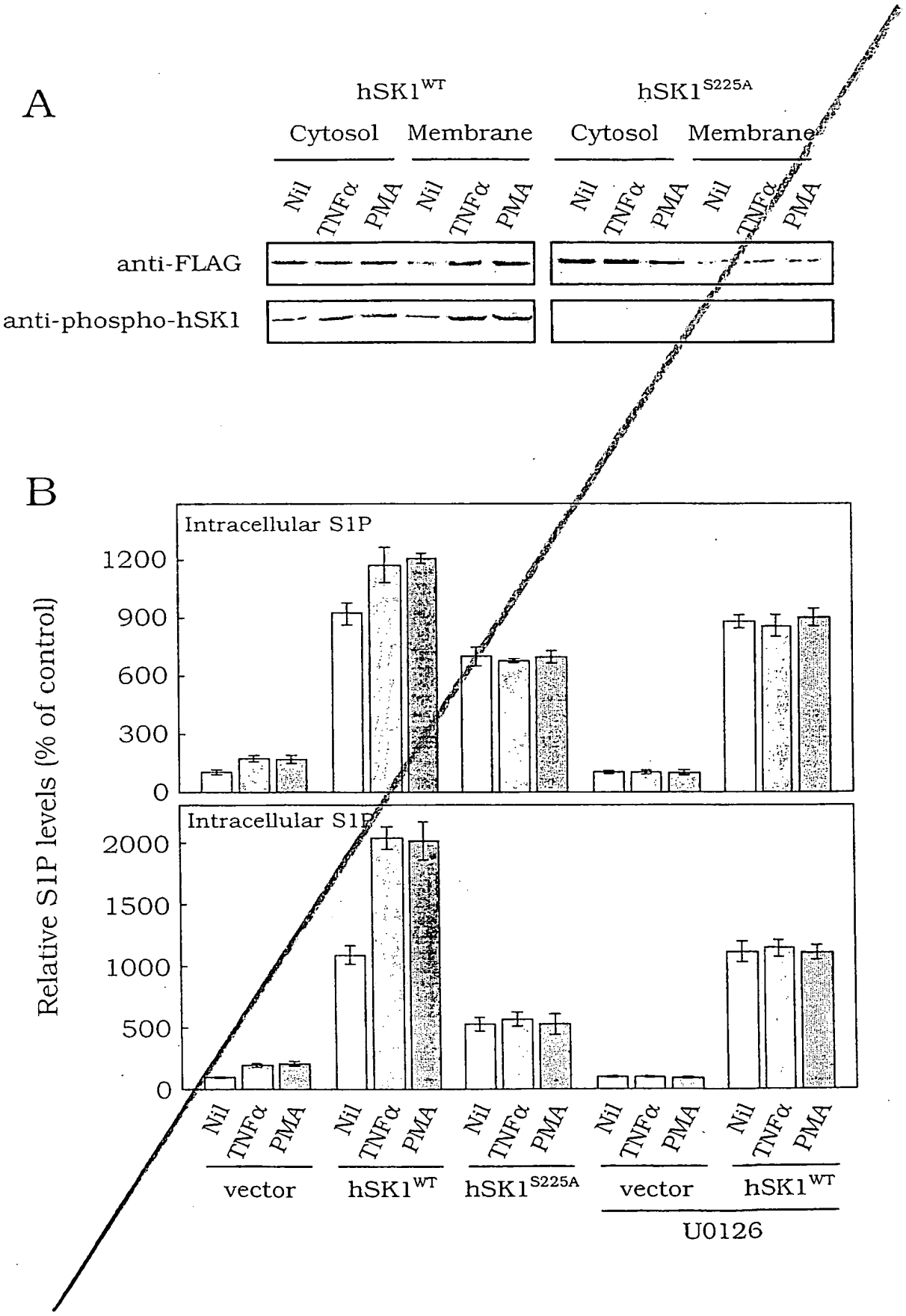


Figure 18